Lipid Biosynthesis in Blood and Egg of Local Hen Fed with Feed Containing Menhaden Fish Oil as Source of Omega-3 Fatty Acids

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Abstract. Menhaden fish oil is commonly used as chicken feed mixture as cheap omega-3 source, plenty and available over the year. Chicken feed containing menhaden fish oil can affect digestion, metabolism and production of meat and egg. The research aimed to evaluate lipid biosynthesis on blood and egg yolk of local chicken fed with feed containing menhaden fish oil as an omega-3 source. The research used 60 hens placed in individual battery cages with four treatments and five repetitions. The treatments were R_0 = control/without menhaden fish oil as omega-3 source; R_1 = with 2.5%; R_2= with 5.0% and R_3 = with 7.5% menhaden fish oils respectively. Data analysis used analysis of variance continued with Duncan's test. The result of the researh showed that the treatments did not signifcantly affect lipid consumption, blood lipid content, blood and yolk cholesterols. However, the treatment significantly affected yolk lipid. The used of menhaden fish oil in feed as an omega-3 source was accumulated in yolk eggs as followed: C18:3 from 0.17% (R_0) to 0.29% (R_2); DHA (C22:6) from 0.87% (R_0) to 3.12% (R_3); EPA C18:2 from 1.05% (R_0) to 1.85% (R_2); EPA C22:4 from 0.77% (R_0) to 0.88% (R_2) respectively. It could be concluded that enhancement of omega-3 content in egg could be achieved through addition of omega-3 sources in feed.

Keywords: egg quality, lipid profile, menhaden fish oil

Abstrak. Minyak ikan lemuru banyak digunakan dalam campuran pakan ayam sebagai sumber omega 3 yang murah, mudah dicari dan terjamin ketersediaannya sepanjang tahun. Pakan ayam dengan penambahan minyak ikan lemuru dapat mempengaruhi terhadap proses pencernaan, metabolisme dan produk yang dihasilkan seperti telur dan daging. Tujuan penelitian untuk mengevaluasi biosintesis lemak pada darah dan kuning telur ayam kampung dengan pemberian minyak ikan lemuru sebagai sumber omega 3. Penelitian menggunakan 60 ekor ayam kampung betina yang ditempatkan pada kandang baterai individual, dengan empat perlakuan dan lima ulangan. Perlakuan terdiri dari: R_0 = kontrol/tanpa minyak ikan lemuru; R_1 = penggunaan minyak ikan lemuru (sumber omega-3) sebanyak 2,5%; R_2= penggunaan minyak ikan lemuru (sumber omega-3) sebanyak 5,0%; R_3 = penggunaan minyak ikan lemuru (sumber omega-3) sebanyak 7,5%. Analisis data dengan variansi serta uji lanjut dengan uji Duncan. Hasil penelitian menunjukkan bahwa perlakuan memberikan pengaruh yang tidak nyata terhadap konsumsi lemak, kandungan lemak darah, kolesterol darah dan kolesterol kuning telur, serta kandungan asam-asam lemak kuning telur tetapi berpengaruh nyata terhadap lemak kuning telur. Penggunaan minyak ikan lemuru dalam pakan sebagai sumber omega-3 akan dideposisikan dalam kuning telur sebesar : C18:3 dari 0,17% (R_0) menjadi 0,29% (R_2); DHA (C22:6) dari 0,87% (R_0) menjadi 3,12% (R_3); EPA C18:2 dari 1,05% (R_0) menjadi 1,85% (R_2); EPA C22:4 dari 0,77% (R_0) menjadi 0,88% (R_2). Kesimpulan penelitian bahwa asam lemak omega-3 dalam telur dapat ditingkatkan dengan pemberian asam lemak omega 3 dalam pakan.

Kata kunci: minyak ikan lemuru, profil lemak, kualitas telur

Introduction

Biological effect of egg containing omega-3 could be classified as functional food due to its beneficial effect to reduce the problem in heart diseases, prostate, breast cancer and immune system failure (Lewis et al., 2000). The omega-3 content in egg merely derives from feed. Grobas et al. (2001) mentioned that chicken strain does not affect the fatty acids composition in egg.

Healthy food should contain high poly unsaturated fatty acids (PUFA) which is safe to heart (Bhatnagar and Durrington, 2003; Erkkila et al., 2003; Meyer et al., 2003). Long chain fatty acids such as omega-3 supplementation in feed will be stored in various body tissues such as meat, egg and muscle (Suarez et al., 1996; Hulbert et al., 2002). Egg containing omega-3 could be produced by feeding the animal with omega 3 enriched-feed 3 namely eico sapentaenoic acid (EPA) and docosa hexaenoic acid (DHA) such as fishmeal and fish oil which could reach 22.08% omega-3 (Hulbert et al., 2002).

Local chicken egg recently becomes the main choice for Indonesian consumers concerning the risk of cholesterol and heart problems. An effort is therefore needed to solve the problem through feed manipulation in egg production to produce eggs containing omega-3.

Materials and Methods

The research used sixty 22-week-old local hens, kept in 60 individual bamboo battery cage measuring 50x33x25 cm, with individual drinking water and plastic feed bowls. The feed ingredients contained menhaden fish oil, palm oil, fish meal, soy flakes, rice bran, corn and mixed minerals (Table 1). The feed composition was formulated based on calculation from the list of feed material composition according to NRC (1994) and analysis result from Animal Nutrition and Feed Laboratory, Faculty of Animal Science, Jenderal Soedirman University.

The treatments of the research consisted of four kinds of feed composition, each treatment consisted of 3 hens and was repeated five times. Experiment was done in vivo, in a Completely Randomized Design (Steel and Torrie, 1994). Data were analyzed using analysis of variance and continued with Duncan's test (Gill, 1978). The feed trials were R_0 = feed without (0%) menhaden fish oil; R_1 = feed with 2.5% menhaden fish oil; R_2 = feed with 5% menhaden fish oil; R_3 = feed with 7.5% menhaden fish oil (Table 1).

The variables examined were 1) lipid profile (feed, egg yolk and blood lipids or blood triglyseride), 2) blood and yolk cholesterols. Lipid analysis was done using soxhlet extraction method (AOAC, 1995), cholesterol and blood serum lipid/triglycerides (CHOD-PA method, using Diasys, Diagnostic System Kits*).* Measurement of blood and egg yolk cholesterol used spectrophotometer at wave length of 500 nm (Tranggono and Setiaji, 1989).

Results and Discussion

Most lipids in poultry feed were in form of fatty cholesterol acids, trigycerides and cholesterols which got into intestine and produce fatty acids and glycerols then developed cyclomicron. Cyclomicron along with protein (lipoprotein) was then absorbed in blood circulation and transformed into VLDL, HDL, LDL and cholesterol. Small amount of lipids was in form of free fatty acids. Lipid metabolism manifestation could be performed as lipid and cholesterol contents in egg. The performance of blood and yolk lipids profile was showed in Table 2 and 3.

Table 2 shows that lipid consumption was 3.88-3.99 g/animal/day. Analysis of variance indicated no significant effect ($P>0.05$) to lipid consumption, blood lipid and cholesterol, and yolk cholesterol, but there was significant effect

(P<0.05) on the yolk lipid. This result indicated that feed added with up to 7.5% menhaden fish oil did not affect the palatability of chicken, digestive processes and lipid metabolism.

Reduction of blood lipid followed by reduction of blood and yolk cholesterols was found at the used of menhaden fish oil in feed up to 2.5% level while 5% level of use increased blood cholesterol. This phenomenon indicated that blood cholesterol was from feed and cholesterol synthesis from fatty acid metabolism.

The use of menhaden fish oil at 5% (R_2) significantly resulted in the least yolk lipid and blood cholesterol contents. Thus, it could be used as indicator that the best concentration to use animal lipid in poultry feed should be 5% (v/w).

Lipid consumed as triglyceride would be hydrolyzed in the digestive system to monoglycerides, free fatty acids and glycerols. Free fatty acids re-esterized into intestine cells before excreted into blood (Linder, 2006). Esterized fatty acids would produce triglyceride, cholesterol and cholesterol ether. However, non-esterized fatty acids would produce free fatty acids (Martin et al., 1985). Essential fatty acids found in cell lipid structure, connected with integrity of mitochondrion membrane structure and found at high concentration in reproduction organs, phospholipids and roled as cholesterol development precursor (Harper et al., 1985). The used of omega-3 rich material in ration could reduce the risk of arteriosclerosis in experiment animals. According to van Elswyk (1997), fish oil could reduce very low density lipoprotein (VLDL) cholesterol and triglycerides contents in cockerel blood.

Lipid and cholesterol concentration in yolk was 9.26-11.25% and 0.17–1.30 g/100g respectively from layers fed whole cia and whole flake sheed (Ayerza and Wayne, 2000). In addition, feeding layers with 5% menhaden fish and 5% palm oils resulted in 95.8 mg/dl blood cholesterol (Iriyanti, 2006).

The effect of omega-3 fatty acid could reduce blood and yolk cholesterols, and influenced egg fatty acids content (Caston and Leeson, 1990; Cherian and Sim, 1991; Jiang et al., 1991; Herber and van Elswyk, 1996; Hammershøj, 1997; Niemiec et al., 1997). Addition of menhaden fish oil up to 1.5% could produce cholesterol 12.56-13.1 mg/g yolk, free fatty acids 0.4±0.07-0.48 ±0.08%, reduction of blood cholesterol total from 223.30±3.63 mg/dl (in control) to 125.83±4.23 mg/dl (Chashnidel et al., 2010). Fish oil could reduce lipogenesis in liver and VLDL secretion (Du and Ahn, 2002; Tabeidian et al., 2005), suppress lipid synthesis (Cortinas et al., 2005), coordinator and regulator of lipid oxidation and fatty acids synthesis (Clark, 2001). Fish oil also controlled hepatic enzymes concentration in glucose metabolism and fatty acids biosynthesis. Those enzymes were glucokinase, pyruvate kinase, glucose-6-phosphate dehydrogenase, citrate lyase, acetyl-CoA carboxylase, fatty acid synthase, stearoyl-CoA desaturase and the D-6 and D-5 desaturase. Omega-3 could reduce triacylglycerols, cholesterol and lipoprotein levels in blood serum (Aydin et al., 2005). It also increased β-oxydation and receptor expression for LDL (Belzung et al., 1993). Result of analysis on fatty acids content was shown in Table 3.

Table 2 showed that higher concentration of menhaden fish oil in ration could produce higher DHA in yolk, but the highest EPA could only be reached by feeding with 5% menhaden fish oil (R_2). It indicated that oil or lipid in poultry feed after metabolism could be deposited in yolk before in adipose cells. As mentioned by van Elswyk (1997) that nutrition manipulation for layers containing omega-3 could be deposed in yolk, feeding with 15g menhaden fish oil/kg of feed was the most palatable by consumer respondents and length of storage time. The content of linolenic

Table 1. Ration composition

Feed ingredients	R_0	R_1	R_2	R_3
Corn	49	40.5	30	27
Rice bran	26	33	40.5	41
Soy flake	14	13	13.5	13.5
Fishmeal	9	9	9	9
Menhaden fish oil	0	2.5	5	7.5
Palm oil	0.2	0.2	0.2	0.2
Lime	2	2	2	2
Premix	0.3	0.3	0.3	0.3
	101.5	101.5	101.5	101.5
Chemical composition				
Protein (%)	17.12	16.85	17.39	17.322
Energy (cal/kg) *	2709.1	2745.45	2757.3	2880.5
Crude fiber (%)	5.015	5.5125	6.105	6.085
Crude fat (%)	6.62	8.42	8.60	6.85
Ca (%) **	2.4547	2.4547	2.4547	2.4547
P (%) **	1.2273	1.2273	1.2273	1.2273

Analysis from Animal Feed and Nutrition Laboratory, Faculty of Animal Science, Jenderal Soedirman University (2011), *)analysis from Animal Feed Material Laboratory (2011), **)values based on NRC (1994). R_0 = feed without (0%) menhaden fish oil; R_1 = feed with 2.5% menhaden fish oil; R_2 = feed with 5% menhaden fish oil; R_3 = feed with 7.5% menhaden fish oil

Table 2. Average of lipid profile in blood and yolk of experimental hens

	R_0	R_1	R_2	R_3
Lipid consumption (g)	3.99	3.97	3.96	3.88
Blood lipid (%)	22.00	16.00	14.00	12.00
Blood cholesterol (mg/dl)	16.2	13.80	10.80	15.60
Yolk lipid (%)	4.40	5.57	3.63	4.16
Yolk cholesterol (g/g)	10.13	8.51	7.20	6.68

R_0 = feed without (0%) menhaden fish oil; R_1 = feed with 2.5% menhaden fish oil; R_2 = feed with 5% menhaden fish oil; R_3 = feed with 7.5% menhaden fish oil

Table 3. Average of fatty acids content in yolk

Fatty acids content in yolk	R_0	R_1	R_2	R_3
C18:2	1.05	1.75	1.85	1.63
C18:3 (linolenic acid)	0.17	0.24	0.29	0.27
C20:3	0.12	0.14	0.16	0.17
C20:6	0.24	0.26	0.27	0.24
C22:4	0.77	0.74	0.88	0.71
C22:6 (DHA)	0.87	2.04	2.74	3.12

R_0 = feed without (0%) menhaden fish oil; R_1 = feed with 2.5% menhaden fish oil; R_2 = feed with 5% menhaden fish oil; R_3 = feed with 7.5% menhaden fish oil

(C18:3), arachidonate (C20:4) acids, and DHA (C22:6) in egg could be increased by feeding with feed material sources containing omega-3 fatty acids and essential fatty acids (Gao and Charter, 2000). The use of other feed materials such as flaxseed, safflower oil, perilla oils, algae, fish oil, plant oil could increase omega fatty acids content in egg (Jiang et al., 1991; Herber and van Elswyk, 1996; Kim et al., 1997; Chae et al., 1998). Feeding with feed containing 6% menhaden fish oil could produce egg containing linoleic acid (C18:3) as much as 0.96 mg/g; EPA (C20:5) 1.41 mg/g; DHA (C22:6) 11.47 mg/g; LA (C18:2) 25.62 mg/g; AA (C20:4) 1.07 mg/g (Kralik et al., 2008). In addition, Laca et al. (2009) reported that omega-3 enriched egg

contained EPA 0.48±0.22 mg/g and DHA 15.91±0.57 mg/g of yolk.

The use of higher level of fish oil in ration could lead to higher omega-3 fatty acids deposit in yolk. Omega-3 fatty acids content in yolk was dominated by DHA as a result from source of fish oil i.e. tuna as reported by Ackman (1982). Domination of omega-3 DHA in yolk was also caused by metabolic transformation of linoleic acid and EPA to DHA (British Nutrition Foundation's, 1994). The mechanism was due to influencial activity of linoleic acid by increasing desaturation. According to Raes et al. (2002) omega-3 and omega-6 synthesis depended on feed's fatty acids profile.

Yannakopoulos et al. (2005) reported that concentration of α-LNA, EPA and DHA in yolk increased during the growth of layers from 22 to 32 weeks old. Feed manipulation using omega fatty acids determined omega-3 content in egg (Yannakopoulos et al., 2005; Sari et al., 2002; Boruta and Niemiec, 2002). Simopoulos (2003) reported that increasing omega-3 PUFA could reach 20 times compared to ordinary feed. Galobart (2002) mentioned fatty acids content in yolk consisted of SAFA 29.83-32.27%, MUFA 38.80-43.36% and PUFA 26.81-29.20%. Schreiner et al. (2004) stated that chicken egg yolk contained fatty acids SAFA 36.12-36.87%, MUFA 47.48% and PUFA 12.55%. Chapman (1980) mentioned that lipid in layer hen was synthesized in liver then transferred to yolk as lipoprotein. Lipoprotein is precursor of yolk lipid, VLDL and the biggest portion of yolk. During the development of chick embryo, the major metabolism was conducted in yolk (Noble and Connor, 1984; Noble and Shand, 1985). The development of embryo was started from desaturate enrichment and carbon chain elongation from linoleic and α-linolenaic acids as PUFA supply (Bordoni et al., 1986; Budowski and Crawford, 1986).

Conclusion

Enhancement of omega-3 fatty acids content in eggs could be achieved through addition of omega-3 sources in feed.

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